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ICMSF methods studies. VI. The influence of
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in dried foods and feeds

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ICMSF methods studies. VI. The influence of selective enrichment media and incubation temperatures on the detection of *Salmonella* in dried foods and feeds^{1,2,3}

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Recovery of *Salmonella* from low-moisture foods was studied using selective enrichment in tetrathionate brilliant green and selenite-cystine broths at 35C and 43C. A total of 1375 samples of dried dairy, soya, and rendered animal by-products were analyzed. All samples were preenriched before selective enrichment. No significant difference in recovery was detected between tetrathionate brilliant green and selenite cystine at either temperature, which is in contrast to our earlier findings with red meats. Pairing of enrichment conditions resulted in the detection of more positive samples than the use of any single enrichment condition.

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On a étudié l'isolement des *Salmonella* à partir d'aliments à faible humidité par enrichissement sélectif. A cette fin on a utilisé les bouillons tétrathionate-vert brillant et sélénite-cystine à des températures d'incubation de 35C et 43C. On a analysé 1375 échantillons déshydratés de produits laitiers, de soya et de sous-produits de consommation d'origine animale. Tous les échantillons ont été pré-enrichis avant l'enrichissement sélectif. Aucune différence significative dans la récupération a été décelée entre le tétrathionate-vert brillant et le sélénite-cystine et ceci aux deux températures d'incubation utilisées; cela diffère des résultats obtenus avec des viandes rouges. Lorsqu'on utilise les conditions d'enrichissement en paires on décele plus d'échantillons positifs que par l'utilisation d'une seule condition, quelle qu'elle soit.

[Traduit par le journal]

A previous communication (2) reported that incubation of selective enrichment broths at 43C resulted in the detection of significantly more salmonellae-positive samples of red meats than did incubation at 35C. The study reported now was undertaken to determine whether incubation at 43C would similarly influence the detection of salmonellae in dried foods and feed ingredients.

Methods and Materials

Samples

Dried dairy, soya, and rendered animal by-products, routinely submitted to this laboratory, were analyzed. The samples were selected from sources known to have a high incidence of contamination with salmonellae. The sample sizes ranged from 25 g to 500 g.

Analytical Procedures

Soya products were preenriched in lactose broth, while rendered animal by-products were enriched in lactose broth containing Tergitol, as previously described (4). Dairy products were preenriched in distilled water containing brilliant green dye (5). All preenrichment cultures were 1:10 dilutions of the product and all were incubated at 35C for 24 h.

After incubation the preenriched samples were mixed thoroughly, and 1-ml aliquots were transferred to duplicate tubes containing 9 ml selenite-cystine broth (Difco) and duplicate tubes containing 9 ml tetrathionate broth (Difco) with added brilliant green dye (5). One tube each of selenite cystine and tetrathionate brilliant green was incubated at 35C for 24 h and the second tube of each was incubated at 43C for 24 h. Incubation at 43C was done in a water bath maintained at $43 \pm 0.01C$.

The selective broths were analyzed for salmonellae as previously described (4).

Results and Discussion

Salmonellae were isolated from 133 of the 1375 samples examined (Table 1). No single medium-temperature combination detected all positive samples. Considering all three product categories, tetrathionate brilliant green broth incubated at 43C detected the most positives,

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TABLE 1

Comparative recovery of salmonellae in dried foods and feed ingredients using tetrathionate brilliant green and selenite-cystine selective enrichment broth at 35C and 43C incubation

Product category	Total aliquots analyzed	Total* aliquots positive	Number of positive aliquots			
			Tetrathionate brilliant green		Selenite cystine	
			35C	43C	35C	43C
Dairy	848	66	52	60	52	52
Soya	332	37	32	33	33	27
Meat, bone, fish meal	195	30	25	25	25	27
Totals	1375	133	109	118	110	106

*Total number of positive aliquots based upon combined results from all four conditions.

i.e. 118. However, Chi-square analysis of the media-temperature variables across or within the three product categories indicated that the differences were not significant.

These results contrast sharply with those we obtained earlier with frozen red meats (high moisture) (2). In that earlier work, incubation of selective broths at 43C resulted in detection of significantly more positive samples than incubation at 35C, with selenite-cystine broth at 43C yielding the highest recovery. Other workers have obtained similar results (1, 3).

The difference between our results for red meats and dried products suggests that the background microflora of the food is an important factor in the isolation of salmonellae. Publications on improved recovery of salmonellae by 43C incubation always involved tests with meat or feces samples, which invariably contain an abundance of competing microorganisms. In contrast, dried foods and feed ingredients such as those tested in the present study have nearly always contained relatively small numbers of microorganisms ($< 10^5/g$), including salmonellae.

Table 2 summarizes the recovery rates for the six possible pairings of temperatures and media. As would be expected, the use of two enrichment systems consistently resulted in the detection of more *Salmonella*-positive samples than the use of a single enrichment system. Table 1 shows that tetrathionate brilliant green broth at 43C detected the greatest number of any single selective enrichment condition. The highest recovery rate of paired media (129 of 133 positive) was obtained with tetrathionate brilliant green broth at 43C combined with the same medium at 35C.

In conclusion, the data do not indicate that one temperature-medium variable is statistically superior to the others. However, it is clearly indicated that at least two individual tubes of selective enrichment medium should be used.

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TABLE 2

Comparative recovery of salmonellae in dried foods and feed ingredients using paired media/temperature combinations

Product category	Number of positive samples for pairs of different media/temperature combinations*					
	TBG-35	TBG-35	TBG-35	TBG-43	TBG-43	SC-35
	TBG-43	SC-35	SC-43	SC-35	SC-43	SC-43
Dairy	64	53	59	64	62	58
Soya	36	35	31	36	32	33
Meat, bone, fish meal	29	27	30	29	28	29
Totals	129	115	120	129	122	120

*Media/temperature combinations: 1, tetrathionate brilliant green/35C; 2, tetrathionate brilliant green/43C; 3, selenite cystine/35C; 4, selenite cystine/43C.

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